washing with RPMI and re-stimulation with irradiated autologous PBMC (2500 rad, T:APC=1:4) plus peptide-Ag (10 μg/ml) for 72 hours. Cytokines (pg/ml) profiles were monitored by immunoassay (ELISA) of supernatants. Each experiment shown is representative of at least three independent experiments. Bars represent mean ± SEM.

On page 81, line 25 through page 82, line 2, please delete the following paragraph:

To assess the effects of RTL pre-treatment on subsequent response to antigen, T cell clones pretreated with anti-CD3 or RTLs were restimulated with APC/peptide, and cell surface markers, proliferation and cytokine production were monitored. RTL pre-treatment had no effect on the cell surface expression levels of CD25, CD69 or CD134 (OX40) induced by restimulation with APC/peptide compared to T cells stimulated with APC/peptide that had never seen RTLs, and there were no apoptotic changes observed over a 72 hour period using Annexin V staining (data not shown).

On page 81, line 25, please insert the following new paragraph:

To assess the effects of RTL pre-treatment on subsequent response to antigen, T cell clones pretreated with anti-CD3 or RTLs were restimulated with APC/peptide, and cell surface markers, proliferation and cytokine production were monitored. T cell clones were cultured at 50,000 cells/well with medium, anti-CD3, or 20 μM RTLs in triplicate for 48 hours, and washed once with RPMI. After the wash, irradiated (2500 rad) frozen autologous PBMC (150,000/well) plus peptide-Ag (MBP-85-99 at 10 μg/ml) were added and the cells incubated for 72 hr with ^{3}H-thymidine added for the last 18 hr. For cytokine assays, clones were cultured with 10 μg/ml anti-CD3 or 20 μM RTL303 or RTL311 for 48 hours, followed by washing with RPMI and re-stimulation with irradiated autologous PBMC (2500 rad, T:APC=1:4) plus peptide-Ag (10 μg/ml) for 72 hours. Cytokines (pg/ml) profiles were monitored by immunoassay (ELISA) of supernatants. RTL pre-treatment had no effect on the cell surface expression levels of CD25, CD69 or CD134 (OX40) induced by restimulation with APC/peptide compared to T cells stimulated with APC/peptide that had never seen RTLs, and there were no apoptotic changes observed over a 72 hour period using Annexin V staining (data not shown).

On page 82, please delete the paragraph on lines 3-6:

As anticipated, anti-CD3 pretreated T cells were strongly inhibited, exhibiting a 71% decrease in proliferation and >95% inhibition of cytokine production, with continued IL-2R (CD25) expression (Table 6; Fig. 25), a pattern consistent with classical anergy (Elder et al., 1994).

On page 82, line 3, please insert the following new paragraph:

a3

As anticipated, anti-CD3 pretreated T cells were strongly inhibited, exhibiting a 71% decrease in proliferation and >95% inhibition of cytokine production, with continued IL-2R (CD25) expression (Table 6), a pattern consistent with classical anergy (Elder et al., 1994). T cells showed a reduced ability to proliferate and produce cytokines after anti-CD3 or RTL treatment, and the RTL effect was antigen and MHC specific. IL-10 was induced only by specific RTLs, and IL-10 production was maintained even after restimulation with APC/antigen.

On page 82, line 15 through page 83, line9, please delete the paragraph:

Clone MR#3-1 showed a 42% inhibition of proliferation when pretreated with 20 μM RTL303, and clone MR#2-87 showed a 57% inhibition of proliferation when pretreated with 20 μM RTL311 (Table 6; Fig. 25). Inhibition of proliferation was also MHC class II-specific, as clone CP#1-15 (HLA-DR7 homozygous donor; MBP85-99 specific) showed little change in proliferation after pre-treatment with RTL303 or RTL311 (Table I). Clone MR#3-1 pretreated with RTL303 followed by restimulation with APC/Ag showed a 25% reduction in IL-2, a 23% reduction in IFN-γ and no significant changes in IL-4 production (Fig. 25). Similarly, clone MR#2-87 showed a 33% reduction in IL-2, a 62% reduction in IFN-γ production, and no significant change in IL-4 production. Of critical importance, however, both RTL-pretreated T cell clones continued to produce IL-10 upon restimulation with APC/peptide (Fig. 25).

On page 82, line 15, please insert the following new paragraph:

a4

Clone MR#3-1 showed a 42% inhibition of proliferation when pretreated with 20 μM RTL303, and clone MR#2-87 showed a 57% inhibition of proliferation when pretreated with 20

μM RTL311 (Table 6). Inhibition of proliferation was also MHC class II-specific, as clone CP#1-15 (HLA-DR7 homozygous donor; MBP85-99 specific) showed little change in proliferation after pre-treatment with RTL303 or RTL311 (Table I). Clone MR#3-1 pretreated with RTL303 followed by restimulation with APC/Ag showed a 25% reduction in IL-2, a 23% reduction in IFN-γ and no significant changes in IL-4 production. Similarly, clone MR#2-87 showed a 33% reduction in IL-2, a 62% reduction in IFN-γ production, and no significant change in IL-4 production. Of critical importance, however, both RTL-pretreated T cell clones continued to produce IL-10 upon restimulation with APC/peptide.

On page 83, line 15 through page 84, line 3, please delete the paragraph:

In the system described herein, anti-CD3 induced strong initial proliferation and secretion of IL-2, IFN-γ, and IL-4 (Fig. 24). Anti-CD3 pre-treated T cells that were restimulated with APC/antigen had markedly reduced levels of proliferation and cytokine secretion, including IL-2, but retained expression of IL-2R, thus recapitulating the classical anergy pathway (Fig. 25). In contrast, direct treatment with RTLs did not induce proliferation, Th1 cytokine responses, or IL-2R expression, but did strongly induce IL-10 secretion (Fig. 24). RTL pretreatment partially reduced proliferation responses and Th1 cytokine secretion, but did not inhibit IL-2R expression upon restimulation of the T cells with APC/antigen. Importantly, these T cells continued to secrete IL-10 (Fig. 25). Thus, it is apparent that the focused activation of T cells through antibody crosslinking of the CD3-chain had vastly different consequences than activation by RTLs presumably through the exposed TCR surface. It is probable that interaction of the TCR with MHC/antigen involves more elements and a more complex set of signals than activation by crosslinking CD3-chains, and the results described herein indicate that signal transduction induced by anti-CD3 antibody may not accurately portray ligand-induced activation through the TCR. Thus, CD3 activation alone likely does not comprise a normal physiological pathway.

On page 83, line 15, please insert the following new paragraph:

In the system described herein, anti-CD3 induced strong initial proliferation and secretion of IL-2, IFN-γ, and IL-4 (Fig. 24). Anti-CD3 pre-treated T cells that were restimulated with APC/antigen had markedly reduced levels of proliferation and cytokine secretion, including IL-